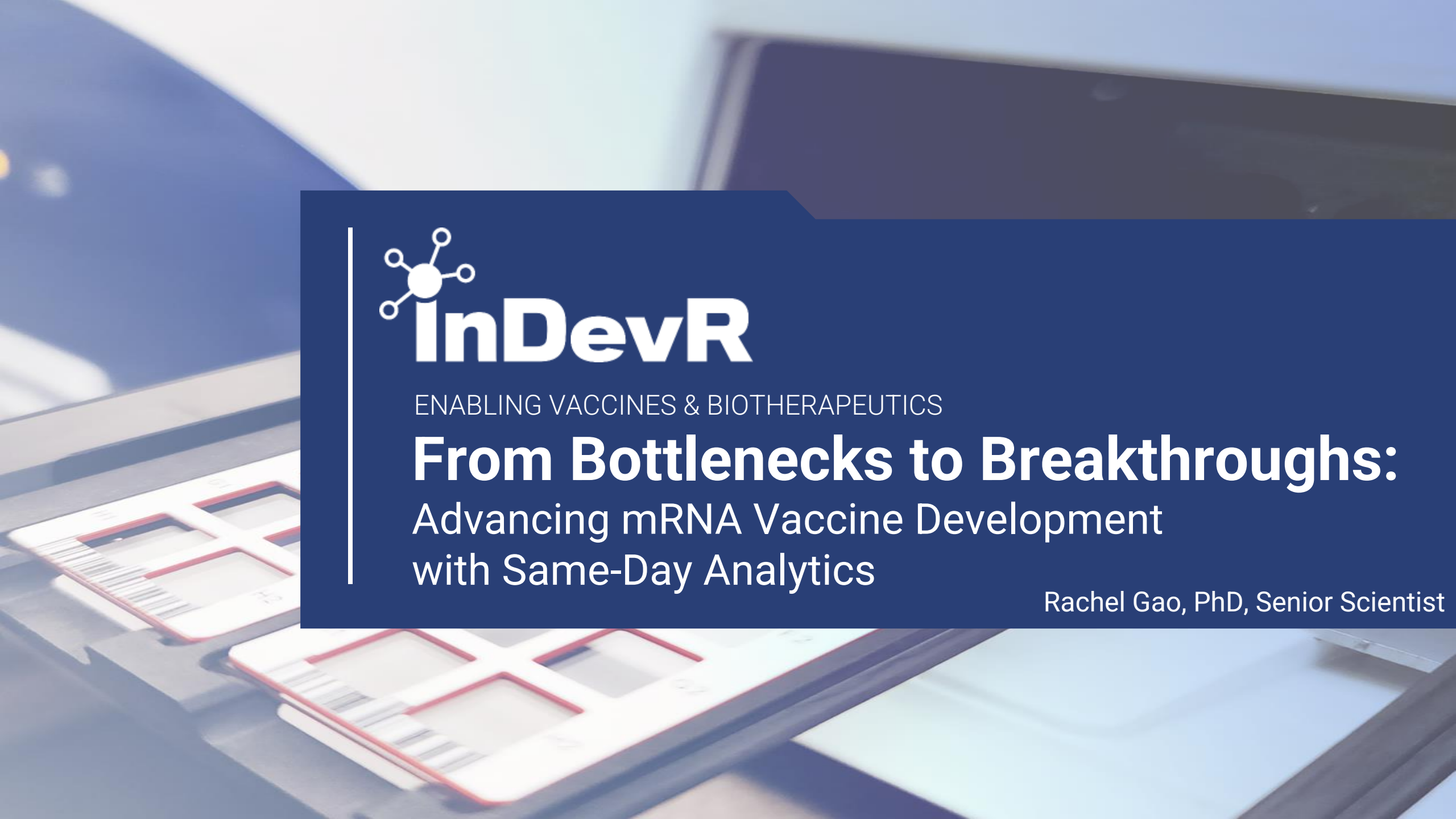


InDevR

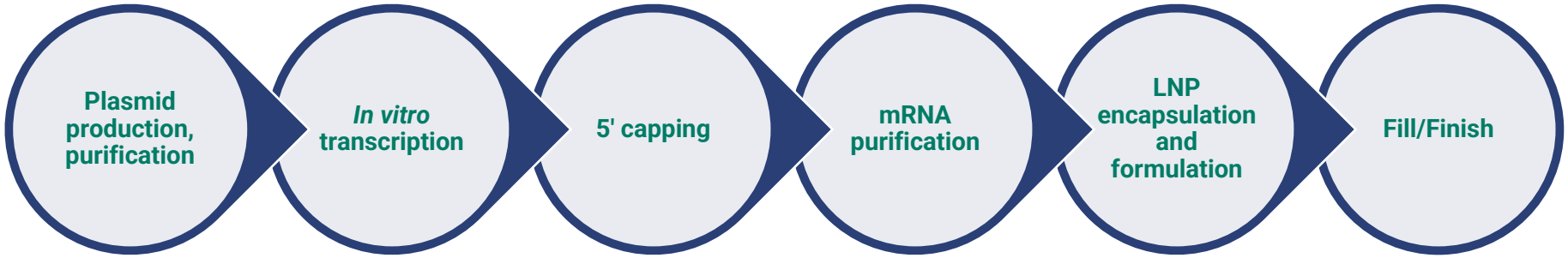
ENABLING VACCINES & BIOTHERAPEUTICS

From Bottlenecks to Breakthroughs:

Advancing mRNA Vaccine Development with Same-Day Analytics

Rachel Gao, PhD, Senior Scientist

mRNA Analytical Development Bottlenecks



Example Areas of Analytical Technology Constraints

Need	**Comprehensive mRNA construct characterization during design, development, and manufacturing**			**Robust cell-based potency assays**	**'Validate-able' Release Assays**
Challenges	**dsRNA, impurities**	**Method complexity, times to result**	**Increasingly multivalent formulations**	**Need Excellent Sensitivity**; **Increasingly multivalent formulations**	**Robust, high ease of use, repeatable, GMP-ready**

InDevR: Next-Generation Vaccine Analytics

Founded in 2003
Boulder, CO

InDevR's mission is to **eliminate analytical development bottlenecks** in vaccine development and manufacturing

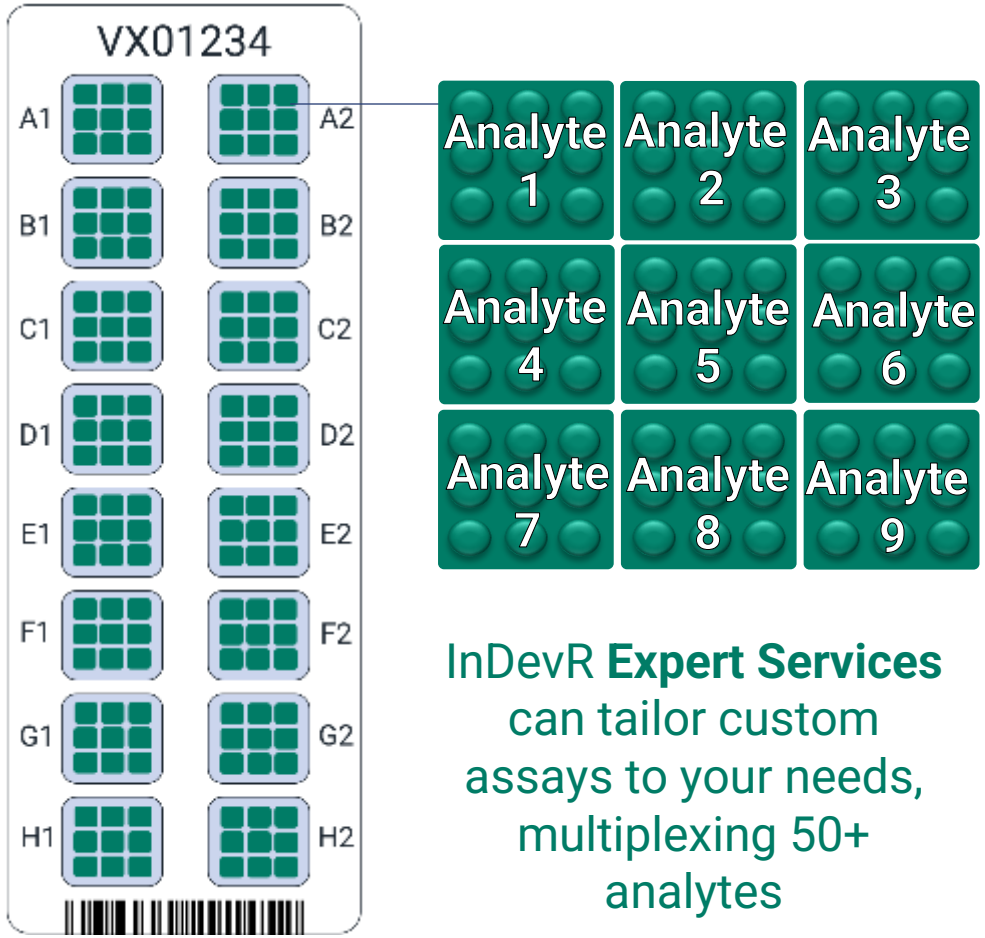
- **2 decades** of streamlining CQA/QC testing for diverse vaccine types (egg-based, cell-based, mRNA)
- **First in industry** to deliver novel strain-responsive off-the-shelf assay kits for **multivalent** influenza antigen testing (HA, NA, NP)
- **Expert Services** division offers tailored solutions for mRNA vaccines as well as traditional vaccines
- Unveiled new cutting-edge facility in Q4 2023 with expanded laboratory capacity

VaxArray Platform Versatility for mRNA Applications

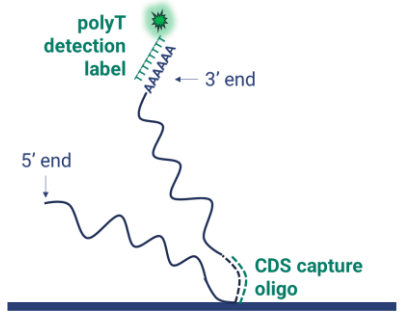
- **Multiplexed Assays Accelerate Analytical Development Time**
- **Standardized Off-the-Shelf Assay Kits Empower Reproducibility**
- **Custom Assay Development Services Available**

Bioprocess	Drug Product Testing	Clinical/Serology
Rapid multiplexed expressed protein quantitation	Extraction-free LNP-mRNA identity and quantity testing	Multiplexed quantification of antibody levels
Naked mRNA construct identity and quantity testing	Rapid multiplexed expressed protein quantitation	
Rapid 5'-capping efficiency assessment		

Single Instrument, Multiple Assay Options

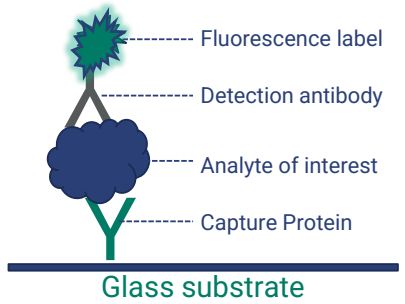


InDevR **Expert Services** can tailor custom assays to your needs, multiplexing 50+ analytes



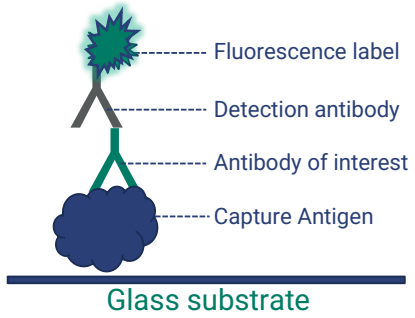
VaxArray mRNA Oligo Assay

- Measure construct-specific mRNA identity and quantity without extraction



VaxArray Antibody Assay

- Measure identity and quantity of protein expression from multiplexed mRNA transfection



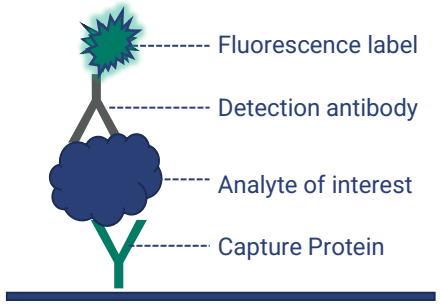
VaxArray Antigen Assay

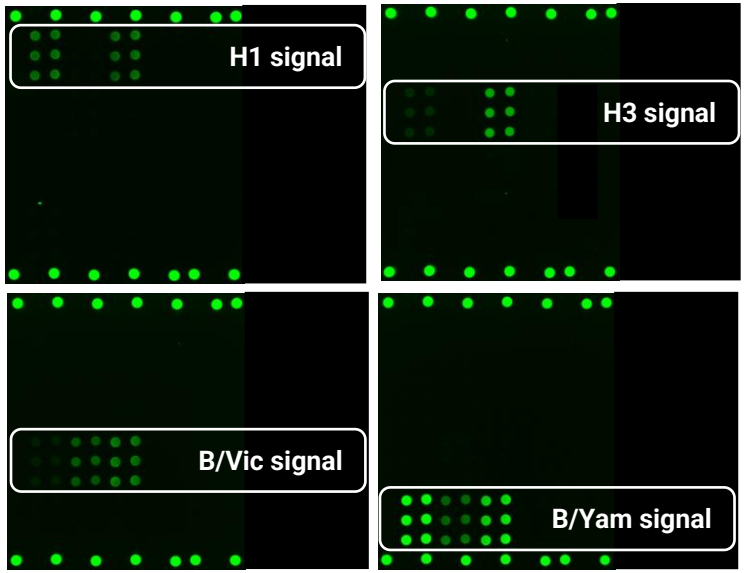
- Measure antibody response from patient serological samples

Applications in mRNA Analytics

In Vitro Potency: Quantify Proteins Post-Transfection

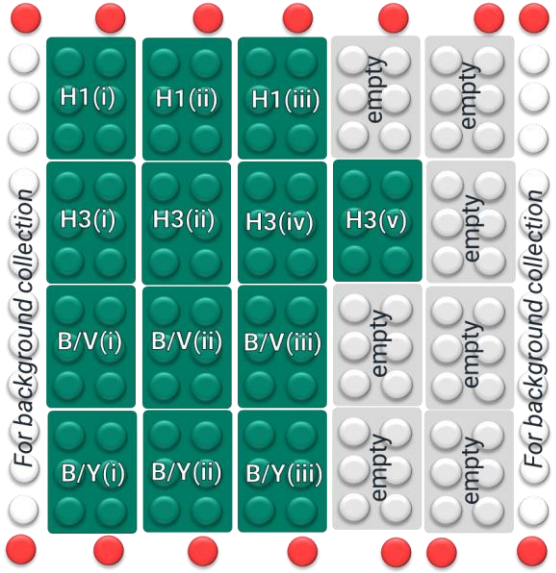
Assess multivalent expressed proteins with high specificity



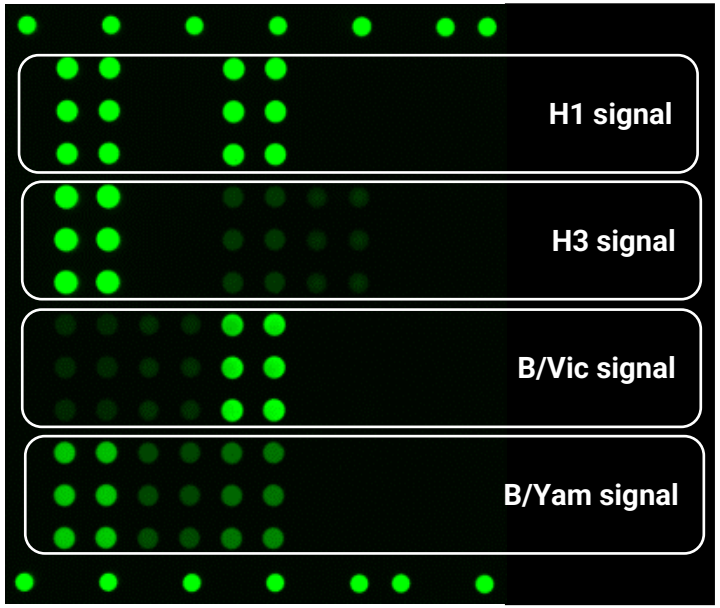


Multiple captures provide reactivity to a broad range of flu strains

Reactivity re-evaluated semi-annually with updated vaccine strains



HA microarray layout



Enables simultaneous quantification of all 4 expressed proteins after quadrivalent mRNA transfection

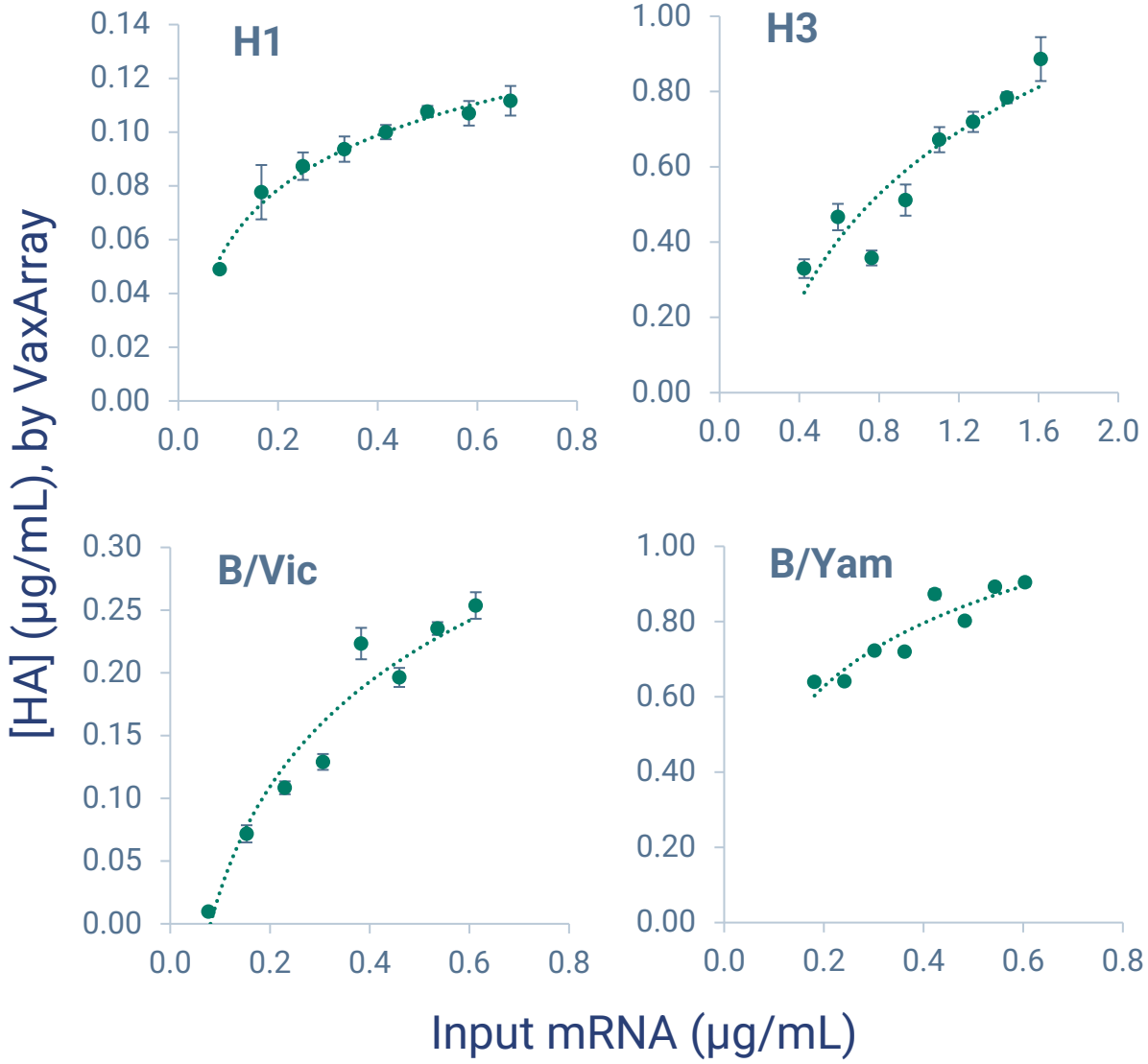
In Vitro Potency: Quantify Proteins Post-Transfection

Evaluate functional protein during optimization of transfection conditions:

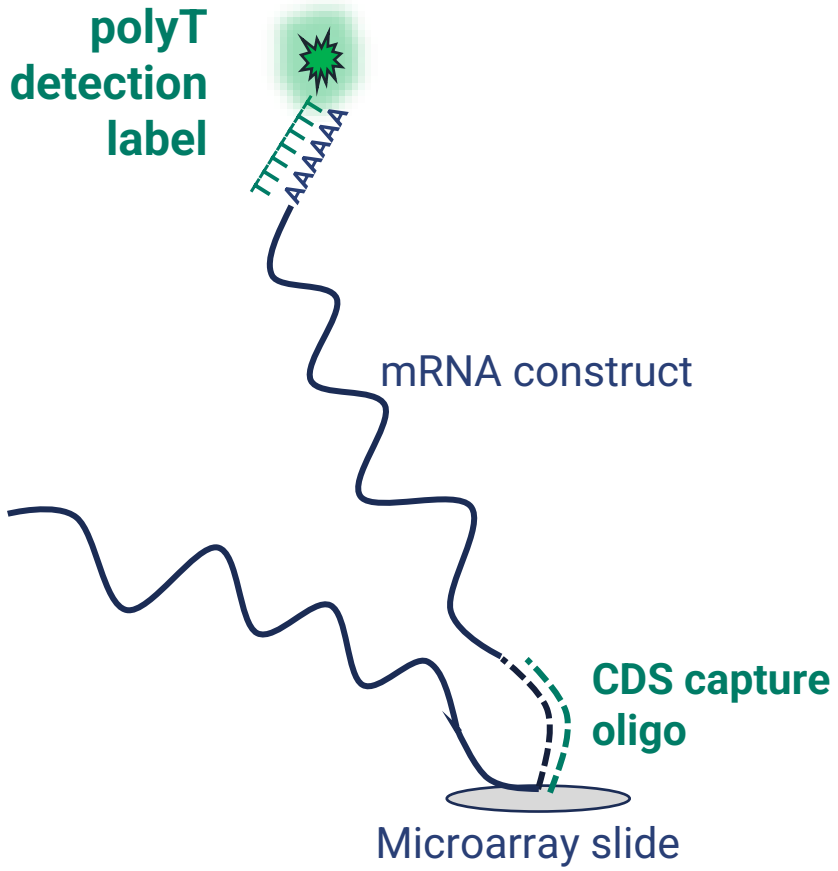
- **Input mRNA concentration**
- **Sequence optimization**
- **Transfection time**
- **Batch to batch**

- HEK293-expressed protein post-transfection with a **4-valent mixture of influenza HA mRNA constructs** measured by VaxArray HA Assay as a function of input mRNA

- As expected, the different proteins exhibit different expression levels



Identity and Quantity of mRNA Constructs



- Identity and quantity of each construct in multivalent mRNA vaccines
- <90-minute time to result
- Capture oligos are designed to target coding regions (CDS) for each construct (ex: each component in a flu vaccine)
- Single detection label for all constructs (custom labels can be designed)
- Works with both naked and LNP-encapsulated mRNA (quick 10-min lysis)
- Can be designed on a custom basis

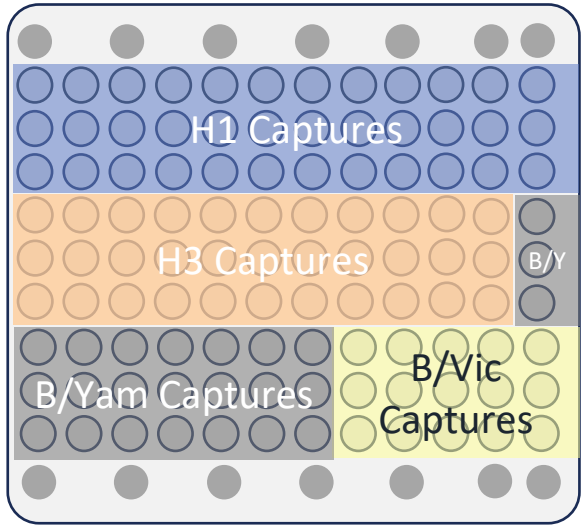
Identity and Quantity for mRNA Constructs

Case Study: Assay for Identity and quantity in 4-valent flu HA mRNA vaccines

- Oligos designed to capture regardless of strain and codon optimization scheme in a single assay

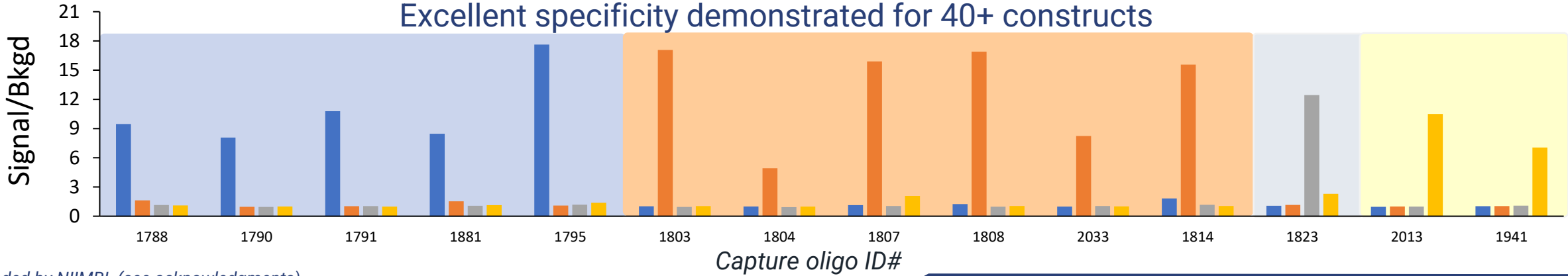
32 capture oligos, with oligos specific for each vaccine component

- Multiple strains over time spanning **11** vaccine seasons
- 3 unique suppliers (unique UTRs)
- 4 unique codon optimization methods



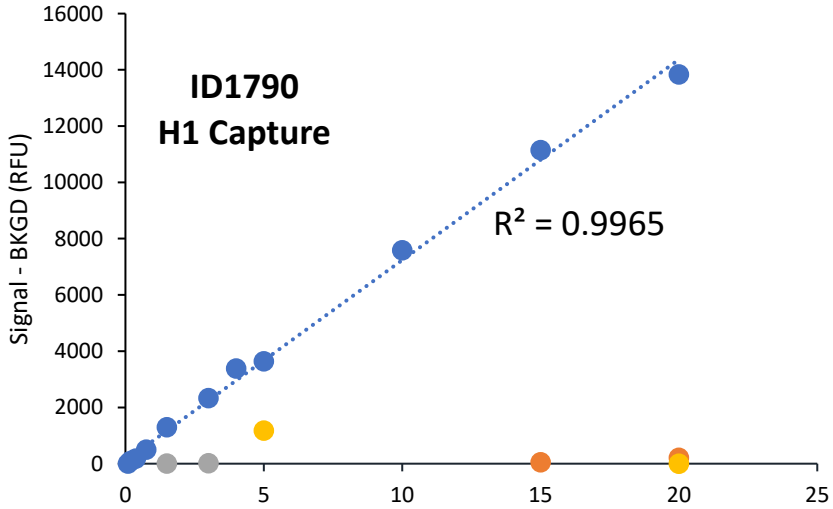
Prototype Array Layout

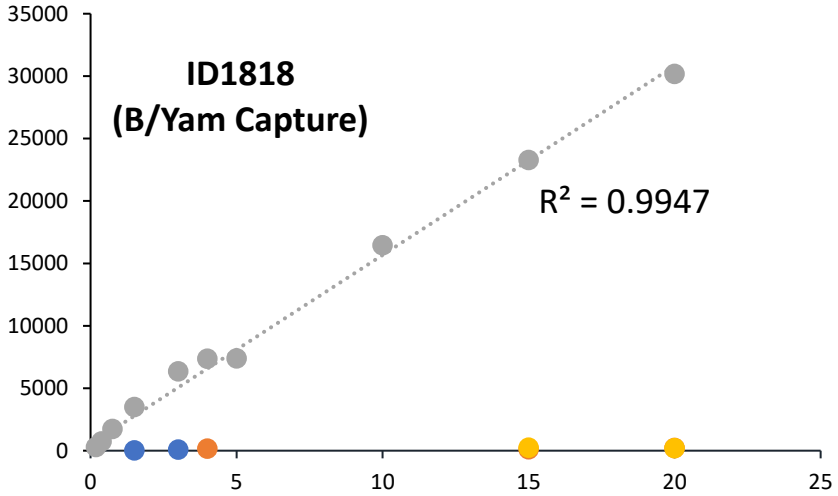
Excellent specificity demonstrated for 40+ constructs

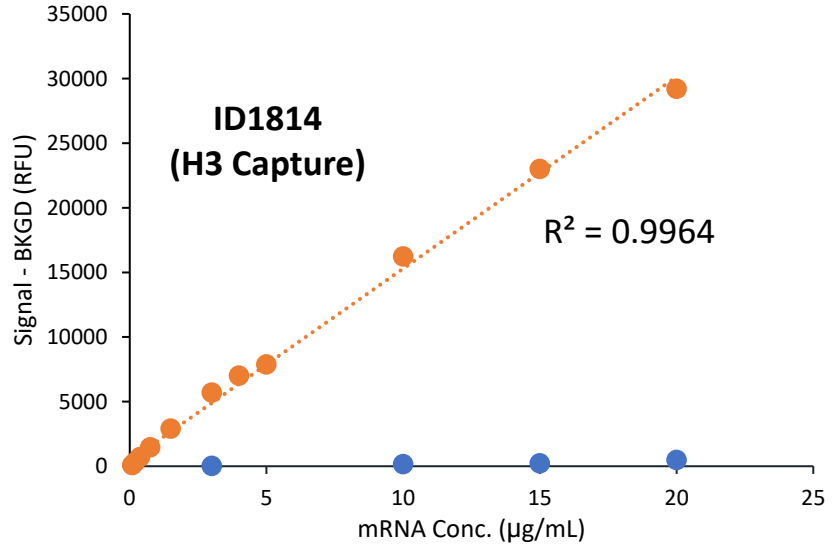


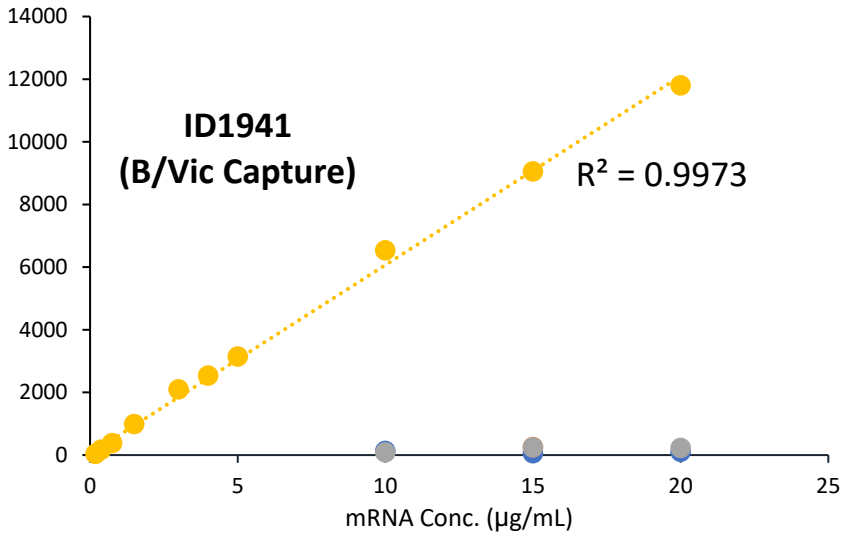
work funded by NIIMBL (see acknowledgments)

Identity and Quantity for mRNA Constructs





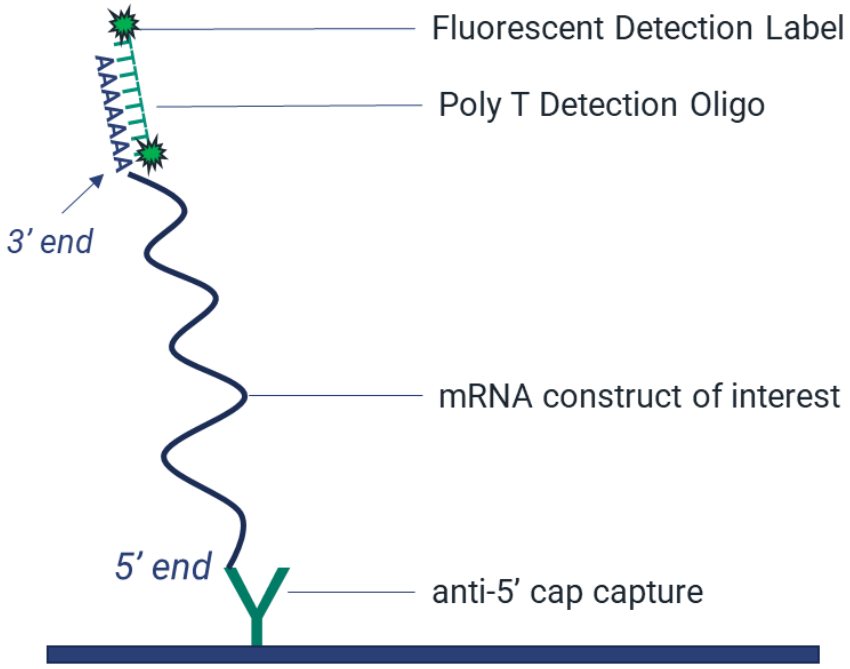




Good linearity over low µg/mL mRNA range

Good specificity: little to no signal on off-target capture oligos

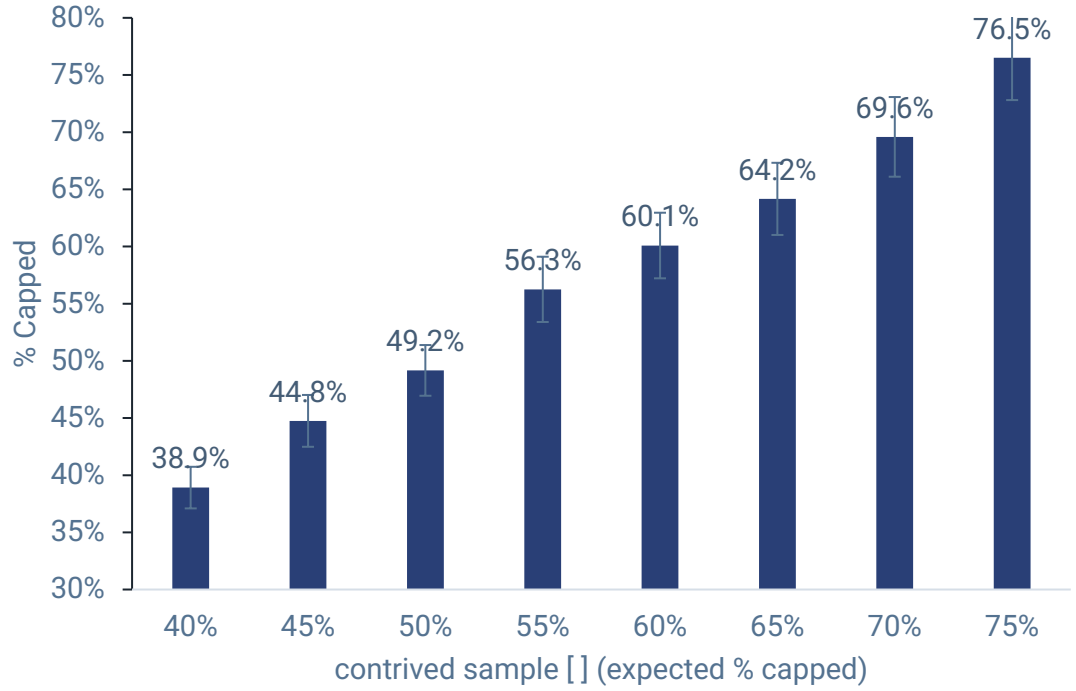
Rapid 5’ Capping Assay for Bioprocess Dev’t



- Capture/detection method is universal to any mRNA construct as long as it has a 5’ cap and a 3’ polyA tail
- < 2-hour time to result
- Assessment of relative capping or quantitative capping efficiency
- For quantitative analysis, user provides characterized matched standard (same construct) if quantification is desired
- Requires an orthogonal measurement of **total mRNA** on the unknown sample by UV-Vis or other method - therefore material must be reasonably pure

Assay Can Differentiate Wide Range of % Capped

- Contrived samples created by mixing uncapped and capped GFP mRNA construct
- Samples differed by 5% capping; run alongside a dilution series of known capped standard



Expected Value	Measured Capping Efficiency (95% CI)	Difference from Expected
40%	38.9 (38.4 – 39.4)%	-1.1%
45%	44.8 (44.1 – 45.3)%	-0.2%
50%	49.2 (48.5 – 49.8)%	-0.8%
55%	56.3 (55.5 – 57.0)%	+1.3%
60%	60.1 (59.3 – 60.8)%	+0.1%
65%	64.2 (63.3 – 65.0)%	-0.8%
70%	69.6 (68.7 - 70.5%	-0.4%
75%	76.5 (75.5% - 77.5)%	+1.5%

- **All samples statistically differentiated (p-value <0.05 and non-overlapping confidence intervals)**
- **8/8 samples were less than 1.5% difference from expected**

Acknowledgments

Funding (Universal influenza HA mRNA assay): This work was performed under a Project Award Agreement from the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) and financial assistance award 70NANB17H002 from the U.S. Department of Commerce, National Institute of Standards and Technology.

Collaborators/Materials Providers

- Dr. Norbert Pardi, University of Pennsylvania, collaborator on NIIMBL funded project
- 5’ capping assay, materials provided and used during development:
  - North Carolina State University/BTEC, Dr. Jennifer Pancorbo
  - Cisterna, Dr. Hari Bhaskaran
  - Aldevron

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